Title: **ACETYLCHOLINESTERASE ASSAY**

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1.0 OBJECTIVE

To perform the aceytlcholinesterase assay.

2.0 HEALTH AND SAFETY

Personnel should wear a labcoat and chemical resistant gloves. Personnel should be aware that acetylcholinesterase inhibiting compounds are used in this assay and due caution should be taken.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

Any employee who routinely works in the laboratory should be capable of performing this task. Training of new staff should be carried out under supervision of an experienced technical employee familiar with this SOP before the employee can work unsupervised.

4.0 REQUIRED AND RECOMMENDED MATERIALS

This section lists the required supplies and equipment:

spectrophotometer	cuvettes
water bath	pipetors
lab coat	test tubes
chemical resistant gloves	ice and ice bucket
assay reagents,buffers, inhibitors	tissue grinder

5.0 PROCEDURE

5.1 ACETYLCHOLINESTERASE ASSAY

1. Label 6 test tubes for each sample in the following manner:
   [Include sample # on each tube]

   3 replicate test tubes "A", "B" and "C"
   1 eserine blank tube "ESE"
   1 tube "50 µl" for protein } DO NOT ADD ANYTHING TO THIS TUBE UNTIL STEP 7.
   1 tube "100 µl" for protein } DO NOT ADD ANYTHING TO THIS TUBE UNTIL STEP 7.

2. Add 1.425 ml of Tris buffer to tubes A, B, C and ESE.

3. Add 15 µl of 100% ethanol to tubes A, B and C.

4. Add 15 µl of 1x10-3 M eserine to ESE.

5. Weigh tissue in tared plastic cups on 5-place balance and prepare at __ mg/ml in Tris buffer (concentration will vary based on tissue).
   a. Homogenize tissue thoroughly, on ice .
   b. Homogenizer used will vary according to tissue type:
      Whole animal - Pro Scientific model Pro 200 motor with a 20 mm x 150 mm stainless steel generator in a plastic vial
      Whole grass shrimp larvae/embryos – glass test tube homogenizer
      Fish brains - glass test tube homogenizer
   c. Other tissue types will be homogenized according to the most appropiate procedure

6. Add 75 µl of homogenate to tubes A,B,C and ESE.

7. Add 50 µl and 100 µl of homogenate to corresponding tubes for protein analysis. Close tube tops with parafilm and freeze for later analysis.

8. Vortex tubes at 2 minute intervals and place in 30°C shaking water bath for 15 minutes.
   a. remove DTNB and ACTH vials from freezer and allow to thaw completely.

9. Begin the following at 2 minute intervals after the 1st tube has incubated for 15 minutes:

a. Add 33 µl of DTNB to cuvette.
b. Add 967 µl of homogenate to cuvette.
c. Add 10 µl of ACTH to cuvette, cover with parafilm, invert to mix.
Wipe sides with a tissue.
d. Place cuvette in spectrophotometer.
e. Press "SECOND FUNCTION" then "100% T/ZERO A".
Wait for beep, then press "RUN".

{Use a clean 967-µl pipette tip for each eserine blank and between samples.}

10. Repeat steps 1-10 for each sample.

11. Label spectrophotometer printout precisely.

5.2 Spectrophotometer and Water Bath Instructions

1. Turn on shaking water bath before starting assay. Shaker should be at lowest setting. Temperature should be at 30°C.

2. Turn on main spec unit, then module, then printer.

3. Make sure "SEL" light is lit (green) on printer or program will not run.

4. Once spec has stabilized, press "A" on module to load program.
Press "NO" on main unit when prompted.

5. See spectrophotometer and water bath manuals for further information.

5.3 Assay Cleanup

1. After the assay is completed, spent homogenate mixture in the cuvettes and test tubes should be flushed down the drain with plenty of water.

2. Place cuvettes in trash.

3. Wash glassware according to its SOP.

6.0 QUALITY CONTROL/QUALITY ASSURANCE

Personnel should adhere to good laboratory practices performing this assay. This procedure should always be performed with proper precautions to minimize personnel exposure to acetylcholinesterase inhibiting compounds.

7.0 REFERENCES

Ellman, G., K. Courtney, V. Andres, Jr. and R. Featherstone (1961) A new and colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88 95.

Fulton, M. (1989) The effects of certain intrinsic and extrinsic variables on the lethal and sublethal toxicity of selected organophosphorus insecticides in the mummichog, *Fundulus heteroclitus* under laboratory and field conditions. PhD. Dissertation. University of South Carolina. 183 pp.

Lethal and sublethal effects of malathion on three life stages of the grass shrimp, *Palaemonetes pugio*. P. Key, M. Fulton, G. Scott, S. Layman and E. Wirth. 1998. Aquatic Toxicology. 40:311-322.

DeWoskin, R.S. 1984. Good laboratory practice regulations: a comparison. Research Triangle Institute, Research Triangle Park, North Carolina. 63 pp.

USEPA. 1979. Good laboratory practice standards for health effects. Part 772 - Standards for development of test data. Fed. Reg. 44:27362-27375, May 9, 1979.

USEPA. 1980. Physical, chemical, persistence, and ecological effects testing; good laboratory practice standards (proposed rule). 40 CFR 772, Fed. Reg. 45:77353-77365. November 21, 1980.